Institut für Molekulare Mechanismen bei Krankheiten
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. med. vet et phil. II Michael Hottiger

Musculoskeletal Research Unit (MSRU)
Leiterin: Prof. Dr. med. vet. Brigitte von Rechenberg, Dipl. ECVS

Arbeit unter wissenschaftlicher Betreuung von
Dr. med. vet. Peter W. Kronen, Dipl.ECVAA

Plasma concentrations of transdermal fentanyl and buprenorphine in pigs (*Sus scropha domesticus*)

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Suzanne Marie Osorio Lujan

Tierärztin aus Portugal

genehmigt auf Antrag von

Prof. Dr. med. vet. Brigitte von Rechenberg, Referentin

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Plasma Konzentrationen nach transdermaler Fentanyl- und Buprenorphinapplikation in Schweinen

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To my husband Ed, my love, my friend, my inspiration.

To my sons and grandson, Esteban, Tomas and Alejandro, the light of my life.

Summary

Objective: Determine the absorption characteristics of transdermal fentanyl and buprenorphine in swine.

Methods: 24 Yorkshire gilts, (± 27.8 ± 2.2 kg), were randomly assigned to different doses of transdermal patches (TP) of fentanyl (50, 75 and 100 μg/h) or buprenorphine (35 and 70 μg/h), once or twice. 13 blood samples were obtained for each TP applied. Plasma concentrations, area under the curve, peak serum concentration (Cmax) and time to Cmax were obtained.

Results: Fentanyl: Cmax were observed at different time points for the first TP: 30 h for 50 μg/h, 6 h for 75 μg/h and 100 μg/h; and for the second TP: 30 h for 50 μg/h; 36 h for 75 μg/h. Buprenorphine: serum concentrations were not detected for the 35 μg/h TP; Cmax was observed at different times for the 70 μg/h patch: 18 h (n = 1), 24 h (n = 3), 30 h (n = 1) and 42 h (n = 1) after application for the first TP and 12 h after the second TP.

Conclusion: Relevant serum concentrations obtained with 75 or 100 μg/h fentanyl TP suggests they could represent an analgesia option for the laboratory pig weighing 25-30 kg. As concentrations of buprenorphine were variable, this study does not support the use of buprenorphine TP in pigs. Consecutive fentanyl or buprenorphine TPs did not provide reliable serum concentrations. Further pharmacokinetics studies and analgesiometric tests in swine are needed to confirm the clinical adequacy of transdermal patches.

Keywords: analgesia, buprenorphine, fentanyl, swine, transdermal patch.

Zusammenfassung

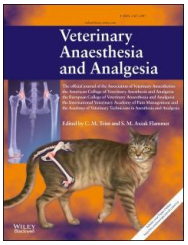
Methoden: 24 Yorkshire-Jungsauen, (27,8 ± 2,2 kg), wurden randomisiert verschiedene Dosierungen Transdermaler Pflaster (TP) mit Fentanyl (50, 75 und 100 µg / h) oder Buprenorphin (35 und 70 µg / h) ein- oder zweimalig zugewiesen. 13 Blutproben wurden für jedes angewandte TP entnommen. Die Plasmakonzentrationen, die Cmax und die Zeit bis Cmax wurden ermittelt.

Ergebnisse: Fentanyl: Cmax wurden zu verschiedenen Zeitpunkten für den ersten TP beobachtet: 30 h für 50 µg/h, 6 h für 75 µg/h und 100 µg/h; Und für das zweite TP: 30 h für 50 µg/h; 36 h für 75 µg/h. Buprenorphin: Serumkonzentrationen wurden für das 35 µg/h TP nicht nachgewiesen; Cmax für den 70 µg/h Patch messbar: 18 h (n = 1), 24 h (n = 3), 30 h (n = 1) und 42 h (n = 1) nach dem ersten TP und 12 h nach dem zweiten TP.

Diskussion: Serumkonzentrationen, die mit 75 oder 100 µg/h Fentanyl TP erhalten wurden, deuten darauf hin, dass sie eine Analgesie-Option für das Laborschwein mit einem Gewicht von 25-30 kg darstellen könnten. Da die Konzentrationen von Buprenorphin hier stark variierten, unterstützen diese Ergebnisse die Verwendung von Buprenorphin TP bei Schweinen nicht. Aufeinanderfolgende Fentanyl- oder Buprenorphin- TPs lieferten keine zuverlässigen Serumkonzentrationen. Weitere pharmakokinetische Studien und analgesiometrische Tests in Schweinen sind erforderlich, um die klinische Wirksamkeit von TPs zu bestätigen.

Stichworte: Analgesie, Fentanyl, Buprenophin, Jungsauen, Transdermales Pflaster

1 Plasma concentrations of transdermal fentanyl and buprenorphine in pigs (Sus scropha domesticus)



Plasma concentrations of transdermal fentanyl and buprenorphine in pigs (*Sus scrofa domesticus*)

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PII: S1467-2987(17)30040-5
DOI: 10.1016/j.vaa.2016.09.002
Reference: VAA 66

To appear in: Veterinary Anaesthesia and Analgesia

Received Date: 7 January 2016
Revised Date: 28 September 2016
Accepted Date: 29 September 2016

Please cite this article as: Lujan SO, Habre W, Daali Y, Pan Z, Kronen PW, Plasma concentrations of transdermal fentanyl and buprenorphine in pigs (*Sus scrofa domesticus*), *Veterinary Anaesthesia and Analgesia* (2017), doi: 10.1016/j.vaa.2016.09.002.

RESEARCH PAPER

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Transdermal fentanyl and buprenorphine in pigs

Plasma concentrations of transdermal fentanyl and buprenorphine in pigs (*Sus scrofa domesticus*)

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Abstract

Objective To determine the absorption characteristics of fentanyl and buprenorphine administered transdermally in swine.

Study design Randomized comparative experimental trial.

Animals Twenty-four Yorkshire gilts weighing mean ± standard deviation 27.8 ± 2.2 kg.

Methods Animals were randomly assigned to different doses of transdermal patches (TP) of fentanyl (50 μg $hour^{-1}$, 75 μg $hour^{-1}$ and 100 μg $hour^{-1}$) or buprenorphine (35 μg $hour^{-1}$ and 70 μg $hour^{-1}$), once or twice. Thirteen blood samples were obtained for each TP applied. Plasma concentrations were determined and the area under the curve, peak serum concentration (Cmax) and time to Cmax calculated.

Results Fentanyl: Cmax were observed at different time points for the first TP application: 30 hours for 50 μg $hour^{-1}$, 6 hours for 75 μg $hour^{-1}$ and 100 μg $hour^{-1}$; and for the second TP application: 30 hours for 50 μg $hour^{-1}$; 36 hours for 75 μg $hour^{-1}$. Buprenorphine: serum concentrations were not detected for the 35 μg $hour^{-1}$ patch; Cmax was observed at different times for the 70 μg $hour^{-1}$ patch: 18 hours (n = 1), 24 hours (n = 3), 30 hours (n = 1) and 42 hours (n = 1) after application for the first patch and 12 hours after the second patch.

Conclusion and clinical relevance A relevant serum concentration obtained with fentanyl TP dosed at 75 μg $hour^{-1}$ or 100 μg $hour^{-1}$ suggests TPs could represent an analgesia option for the laboratory pig weighing 25-30 kg. As concentrations of buprenorphine were variable, this study does not support the use of buprenorphine TP in pigs. Consecutive fentanyl or buprenorphine TPs did not provide reliable serum concentrations. Further pharmacokinetics studies and analgesiometric tests in swine are needed to confirm the clinical adequacy of transdermal patches.

Keywords analgesia, buprenorphine, fentanyl, swine, transdermal patch.

Introduction

The domestic pig (*Sus scrofa domesticus*) has an important place as a model in biomedical research. Many research experiments involve potentially painful interventions, and although pain assessment in pigs has been the subject of several publications (Gregoire et al. 2013; Nalon et al. 2013; Castel et al. 2014; Bradbury et al. 2016), those regarding pain control and analgesic efficacy under experimental research conditions are scarce. The evaluation of pain in animals can be challenging, but it is accepted that nociception during surgical procedures (e.g. thoracotomies) is similar between animals and humans (Livingston 2010; Gigliuto et al. 2014). Therefore, pain control is an animal welfare issue and should be addressed in every scientific project involving animals (Voipio et al. 2008; Kilkenny et al. 2010; Hawkins et al. 2011; National Research Council 2011).

Opioids such as morphine, fentanyl and buprenorphine are commonly used to control pain in veterinary species. Fentanyl is a highly lipophilic pure μ-receptor agonist, that can be administered transdermally (by patches or transdermal solution), by mucosal absorption, and injectable routes [intramuscular (IM), subcutaneous (SC), intravenous (IV), and neuraxial]. Parenteral administration produces a rapid onset but a short duration of action in most animal species (Lamont & Mathews 2007), with a reported effect of less than 4 hours in swine (Swindle 2007). Buprenorphine has been used for many years for treatment of moderate to severe pain in humans. It is thought to be a highly lipophilic partial μ-receptor agonist, κ-receptor antagonist (Johnson et al. 2005; Cowan 2007), with a longer half-life than both fentanyl and morphine. In veterinary practice, parenteral buprenorphine has been used for several decades, and with the exception of rare side effects in a small number of animals, it has been proven safe and efficacious (Roughan & Flecknell 2002; Coulter et al. 2009). However, maintenance of analgesia

with these drugs requires repeated injections, imposing an increased work load for personnel, recurring stress for the animal and often results in discontinuity of overnight pain relief. The highly liposoluble characteristics of fentanyl and buprenorphine favour their use in transdermal administration. Although differences between species and even body sites can be expected, transdermal patches (TP) could be an analgesic option for experimental procedures, reducing many of the limitations of injectable drugs, while allowing good analgesia for a sustained time (Riviere & Papich 2001; Mills et al. 2004). This delivery system is designed to slowly deliver a constant drug dose through the skin, reaching therapeutic concentrations after several hours and lasting up to three days. TPs are current practice in human medicine and, even though controversies exist regarding a proven effect in animals (Hofmeister & Egger 2004; Egger et al. 2007; Brayden et al. 2010), TPs, especially fentanyl, are popular in veterinary medicine, being a recommended method for pain control in many institutions. Recommendations for use of fentanyl TP in pigs are based on few studies, however, wide inter-individual variations and variable magnitudes and times to peak plasma concentrations have been reported (Harvey-Clark et al. 2000; Wilkinson et al. 2001; Malavasi et al. 2005, 2006; Royal et al. 2013). A single publication on use of transdermal buprenorphine in Göttingen minipigs (Thiede et al. 2014) was found in a literature search. In view of the paucity of published studies on transdermal buprenorphine and the reported unpredictability of transdermal fentanyl in pigs, the possibility of species variability and the potential severity of surgical and anaesthetic techniques used under experimental conditions, the need for further studies seemed indicated.

The aim of this study was to determine the absorption and concentration of fentanyl and buprenorphine administered transdermally in pigs. Our hypothesis was that application of a

fentanyl or buprenorphine TP will result in detectable and clinically relevant serum concentrations in pigs postoperatively for 3-4 days without the need for supplementation.

Materials and methods

This study was performed between September 2006 and September 2007. The protocol was approved by the Ethics Committee of the University Hospital in Geneva, Switzerland and the local and Federal Swiss Veterinary Office (no. 31.1.1043/3088/3), according to European Community Directives, Federation of Laboratory Animal Science Associations (FELASA) and Swiss Federal Regulations.

Animals and housing

Good health status of 24 Yorkshire gilts, weighing [mean ± standard deviation (SD)], 27.8 ± 2.2 kg was confirmed by physical examination by a veterinarian upon arrival to the animal facilities at the University Hospital, Geneva, Switzerland. The animals were housed in groups of three. Water was supplied *ad libitum* and food (Nutriporc growing pigs 25-100 kg feed; Provimi-Kliba AG, Switzerland) was provided twice a day, according to their weights. The light:dark cycle was 12 hours of light and 12 hours of darkness, with a relative humidity of 38% and a temperature of 22 ºC. Wood blocks and hanging chains were supplied for enrichment. The acclimatization period was of three consecutive days. In order to familiarize the animals with the procedures and to the handler, the boxes were visited at least three times a day, with the handler progressively approaching the animals. The protocol included sitting in the cage for up to 30 minutes, slowly progressing to a closer contact. After one day, the observers were able to touch the pigs and mimic the blood sampling procedure.

Study design and protocol

The study was a prospective randomized comparative experimental trial; the investigators were not blinded. Randomization was achieved by drawing pieces of paper out of a box, to assign each animal an opioid and the number of TPs. Opioids administered were fentanyl TP (Durogesic, Janssen-CILAG, Switzerland); n = 12) at 50 (n = 6), 75 (n = 3) or 100 µg hour^{-1} (n = 3) and buprenorphine TP (Grünenthal GmbH, Germany, n = 12) at 35 (n = 6) or 70 µg hour^{-1} (n = 6) (Fig. 1). Sixteen of the 24 animals were administered a second sequential application of the lower dosed patches (50 or 75 µg hour^{-1} for fentanyl and 35 or 70 µg hour^{-1} for buprenorphine; Second series, SS). The second TP was applied 36 hours after discarding the first TP. In an attempt to relate potential painful behaviours with drug doses and serum concentrations, 14 of the 16 gilts that were administered a second patch underwent ovariectomies, before the application of the second TP (Fig. 1).

Anaesthesia and surgical procedures

All anaesthetic protocols were the same. After 12 hours of fasting, the animals were premedicated with xylazine (2 mg kg^{-1}; Rompun; Bayer Healthcare LLC, KS, USA) and ketamine (15 mg kg^{-1}; Narketan, Vetoquinol Ltd, UK) IM. Anaesthesia was induced 20-30 minutes later by mask with isoflurane 5% (Forene; Abbott GmbH & Co., Germany), followed by endotracheal intubation with a 6.0 mm silicone tracheal tube (Hi-Contour; Mallinckrodt Pharmaceuticals, Ireland). Anaesthesia was maintained with isoflurane in 40% oxygen using a vaporizer setting of 1.5-2.0%, and animals were mechanically ventilated with a volume-controlled ventilator (Siemens Servo ventilator 900D; Siemens-ELEMA AB, Sweden).

Cefuroxime (750 mg; Zinacef; GlaxoSmithKline, Switzerland) was administered IV 15 minutes prior to the skin incision. Fentanyl (30 μg kg^{-1} $hour^{-1}$; Syntenyl; Sintetica SA, Switzerland) was infused only during the catheter insertion, and lactated Ringer's solution (10 mL kg^{-1} $hour^{-1}$; Ringerfundin; B. Braun Melsungen AG, Germany) was infused IV during all surgeries.

Twenty-four hours before the first TP application, with the animal under general anaesthesia, a 16 gauge, 20 cm, single lumen catheter (Central Venous Catheterization Set CV-04301; Arrow International, Germany) was inserted into the external jugular vein. The jugular vein was exposed through a 5 cm lateral incision in the neck, the catheter inserted 2-3 cm and fixed with a purse string suture to the vessel wall. A subcutaneous tunnel was blunt dissected so that the catheter passed under the skin to exit laterally in the neck area and the external portion (approximately 5-6 cm) was sutured dorsally to the skin in the interscapular area. After implantation, aspirin (25 mg kg^{-1}; Aspégic; Sanofi-Aventis, Switzerland) was administered once daily in the food to prevent clotting. The catheter was expected to last for the duration of the study and was kept in place with a postoperative barrier dressing (OPSITE; Smith & Nephew, UK) and a cotton mesh extending circumferentially from the head to mid-trunk.

The 14 ovariectomies were performed before the second TP application by the same surgeon, under aseptic conditions and general anaesthesia. Fentanyl infusions were not used during this procedure. One of the ovaries was exposed through a ventral midline incision and extracted after ligation of the ovarian vessels. The animals were observed 4 times a day for signs of pain (Appendix 1), in which case rescue analgesia was planned, with IM buprenorphine, 0.05 mg kg^{-1}, twice daily (Lamont & Mathews 2007).

Patch application

An area of interscapular skin of at least 10 cm^2 (or larger depending on the size of the patch to be applied) was washed and the hair clipped, care taken not to cause bleeding or irritation. Twenty-

four hours after this procedure, the first TPs were applied to the conscious animal on the prepared area, close to the catheter hub, and covered with a barrier dressing and cotton mesh as described earlier. Thirty-six hours after the removal of the first patch, a second patch was applied in some animals, following the same protocol for the skin preparation. TPs were applied immediately after surgery or to the conscious animal for those who did not undergo ovariectomy.

Daily observation and blood sampling schedule

All animals were observed for postoperative pain assessments for 10 minutes every 6 hours, before obtaining the blood samples. Behaviours were recorded and used in a pain assessment score (Harvey-Clark et al. 2000; Malavasi et al. 2006), comprising general behaviour and activity, gait, ambulatory activity, vocalizations, local signs of pain (determined by clinical examination and palpation, at the surgical site and in the area of application of the patch), presence of vomit and defaecation characteristics (Appendix 1). Body weights were also registered on days 0, 7 and 12.

Blood was collected for measurement of drug concentrations beginning 24 hours after the venous catheter insertion immediately before First Series TP (Time 0). The first 10 samples were collected every 6 hours and the last 3 every 12 hours, that is before (0) and at 6, 12, 18, 24, 30, 36, 42, 48, 54, 66, 78 and 90 hours after TP application. The sampling intervals were the same after Second Series TP application, a lapse of 36 hours passed between the removal of the first TP and the application of the second TP. In summary, 13 blood samples were collected during the FS and 26 blood samples were collected from pigs assigned to the FS and SS (Fig. 1).

At each blood collection, the priming volume of the catheter and injection cap (0.6 mL) plus one more mL was withdrawn and discarded. Five mL of blood were then withdrawn and placed in dry sterile tubes (no EDTA or citrate). The catheter was then flushed with saline solution 0.9% and primed with 0.6 mL of heparinized saline (300 UI mL^{-1}). Blood samples were refrigerated

(8ºC) until blood clotted, then centrifuged at 30 000 ***g*** for 10 minutes and the serum harvested and frozen at -20 ºC until analysis.

Fentanyl and buprenorphine analysis

Concentrations of fentanyl and buprenorphine in plasma were determined by Liquid Chromatography-Mass Spectrometry (LC-MS-MS) (Agilent HP1100; Agilent Technologies, Germany) coupled to an ion trap mass spectrometer (Esquire 3000+; Bruker Daltonics, Germany). Quality control (QC) concentrations were at 0.1, 1 and 3 ng mL^{-1} corresponding to 2 × Limit of Quantitation (LoQ), 50% and 80% of the calibration curve. Quantification was performed in positive mode using the following transitions: 468.4→55.0 /472.4→59.0 for buprenorphine/internal standard (10 ng mL^{-1} of buprenorphine-d4) and 337.0→187.8/ 342.0→187.8 for fentanyl/internal standard (10 ng mL^{-1} fentanyl-d5). An extraction with chlorobutane was performed after plasma was basified with 10 µL of triethylamine. Separation was carried out in C18 XTerra column (5 µm × 2.1 mm × 50 mm, Waters Corp, USA) at 0.3 mL $minute^{-1}$ under a gradient mode. Along with the unknown samples, duplicate quality control (n = 3) and standards samples (n = 6) were prepared using blank plasma spiked with fentanyl or buprenorphine covering the expected concentration range were processed. The standard curves were obtained by weighted least-squares regression (weighting=1 X^{-1}) of the measured peak area *versus* the analyte concentrations. The standard curves were then used to calculate concentrations of the analytes in unknown and QC samples. The analytical method was fully validated and showed good performances in terms of accuracy (<20%) and precision calculated as intra-assay precision and inter-assay precision (<15%). LoQ was 0.05 ng mL^{-1} for both fentanyl and buprenorphine.

Statistical analyses

The study was planned to have six pigs per group for the first and second patch application for the lower dosed patches (50 µg $hour^{-1}$ fentanyl and 35 µg $hour^{-1}$ buprenorphine). This sample size would ensure 80% and 90% powers at 5% significance if the mean between-group difference is respectively 1.8 and 2.1 times the common standard deviations of the outcome. The other groups were determined in an attempt to evaluate the absorption rates of different dosed patches: a group of six pigs was allocated to the 70 µg $hour^{-1}$ buprenorphine patch and three animals per group for 75 µg $hour^{-1}$ and 100 µg $hour^{-1}$ fentanyl patches. Two pigs were included in a SS group, for the 75 µg $hour^{-1}$ fentanyl patches and 70 µg $hour^{-1}$ buprenorphine each (Fig. 1).

Profiles of blood concentration of fentanyl and buprenorphine were summarized with area under the curve (AUC), peak serum concentration (Cmax) and time to peak concentration (Tmax). AUC was calculated using the trapezoidal rule. Only AUC_{0-54} hours were compared using analysis of variance (ANOVA) for the first series and AUC_{0-66} hours for the second series, since some pigs did not have blood samples drawn beyond this interval. Mean ± SD of these parameters were calculated for both fentanyl and buprenorphine. Analysis of variance was used to compare these parameters (AUC, Cmax and Tmax) between the different dose groups. Separate analysis was done for the periods of the first and second patch. Non-parametric analysis of variance was performed for Cmax and Tmax when, due to small sample, size normality assumption did not appear to be valid. Kruskal-Wallis test was used to test the null hypothesis of three dose groups under the same distribution. Pair-wise comparisons were performed using Wilcoxon Rank sum test. All calculations were performed using the Statistical Analysis System program (SAS Institute, NC, USA).

Results

Fentanyl analysis

Twelve pigs were administered a first application of either 50 µg hour^{-1} (n = 6), 75 µg hour^{-1} (n = 3) or 100 µg hour^{-1} (n = 3) fentanyl TP. Serum concentrations of fentanyl were detected for the three patch strengths. The application of a second patch (50 µg hour^{-1}, n = 6; 75 µg hour^{-1}, n = 2) in eight animals also provided detectable serum concentrations, but in lower range than the first patches. Cmax were higher for the first 50 and 75 µg hour^{-1} patches applied than for the second corresponding patch (Table 1). AUC was higher after TP 75 or 100 µg hour^{-1} than after 50 µg hour^{-1} for the first and second patches (Fig. 2). Some sample results are missing because the catheters became dislodged, therefore, to include the maximum number of samples in the analysis, the AUC 0-54 hours was calculated for the first patch and AUC 0-66 hours for the second patch. In the first series, differences between TP 50 µg hour^{-1} and 75 µg hour^{-1} were significant (p = 0.0017) and between TP 50 µg hour^{-1} and 100 µg hour^{-1} (p = 0.0006) (Fig. 2). There was no significant difference in AUC between TP 75 and 100 µg hour^{-1} (p = 0.24). Individual profiles of serum fentanyl concentrations showed a wide variation between animals, and between series 1 and 2 (Fig. 3).

Buprenorphine analysis

Serum concentrations of buprenorphine were not detected in any of the six animals administered the lower buprenorphine TP (35 µg hour^{-1}), either for the first or second application. The remaining six animals were administered one (n = 6) or two sequential (n = 2) higher dose buprenorphine TP (70 µg hour^{-1}) patches (Fig. 1). The first TP resulted in a large inter-individual variability for concentration, distribution and magnitude Fig. 4), with Tmax varying between subjects: 18 hours (n = 1), 24 hours (n = 3), 30 hours (n = 1) and 42 hours (n = 1) after application (average 0.3 ± 0.2 ng mL^{-1} at 33 hours). Great variability in serum concentrations (0.38-0.63 ng mL^{-1}) were measured in the two pigs that had a second buprenorphine TP (70 µg hour^{-1}) applied. There were residual serum concentrations of buprenorphine from the first patch applied (0.12-0.17 ng mL^{-1}). Cmax was

attained 12 hours after application and concentrations rapidly decreased after 18 hours.

Daily observations, blood sampling and patch application

The acclimatization period for the animals facilitated handling and daily observations. The indwelling catheters facilitated the blood sampling process, however not all remained patent for the entirety of the study. The average patency was 11.3 ± 1.6 samples in the FS and 25.2 ± 2.2 samples in the SS. Failure of blood collection was the result of different circumstances. Two catheters were pulled out, although no bleeding or hematoma were observed. Two other catheters were obstructed by blood clots and attempts to restore patency were unsuccessful. No infections or local lesions were observed at any of the catheter insertion sites and none of the animals showed signs of local pain on palpation. The exterior injection hubs remained secure and clean and were easy to access.

All TPs remained appropriately fixed to the interscapular skin, and were effectively protected by the barrier dressing. No skin irritation or sign of discomfort was observed. The larger buprenorphine TP (70 µg $hour^{-1}$), with a contact area of 50 cm^2, was more difficult to apply in the study pigs, particularly in the awake animal. The application of the fentanyl TP was simple for the three doses, as the size of the larger patch (42 cm^2 for the 100 µg $hour^{-1}$) was easily accommodated.

Pain assessment

Defaecation occurred normally and no depression, hyperexcitability, aggression or other opioid or pain related side or adverse effects were observed. The behaviour registered for the animals that underwent ovariectomies did not differ significantly from those not ovariectomized. No rescue analgesia was administered. Weight increased in all animals, with mean weights of 27.8 ± 2.2 kg; 30.7 ± 2.9 kg and 31.3 ± 3.3 kg on days 0, 7 and 12, respectively.

Discussion

The serum concentrations measured after application of fentanyl TPs to pigs showed the presence of fentanyl for all three patch strengths (50, 75 and 100 µg $hour^{-1}$), both with the first and the second application of a patch. Plasma concentrations obtained in the present study for the 50 µg $hour^{-1}$ TP concur with those reported previously by several authors. Harvey-Clark et al. (2000) studied the efficacy of 25 (n = 4) and 50 µg $hour^{-1}$ (n = 2) TP of fentanyl in swine with comparable weight to those in the present study, with only the 50 µg $hour^{-1}$ patches providing concentrations above the 0.5 ng mL^{-1} human threshold that the authors used for comparison. Large inter individual variations in the observed plasma concentrations were reported, but the authors concluded nevertheless that the 50 µg $hour^{-1}$ TP can provide adequate basal analgesia (Harvey-Clarke et al. 2000). Malavasi et al. (2005) studied the effect of 50 µg $hour^{-1}$ fentanyl TP on the activity of growing pigs (average weight 25.4 ± 5.2 kg). Serum fentanyl measurements after 24 hours were 0.27 ± 0.11 ng mL^{-1} (n = 8) and 0.47 ± 0.40 ng mL^{-1} (n = 8). They concluded that transdermal fentanyl can be absorbed, but the wide variation of serum concentrations renders the use of fentanyl patches unpredictable and potentially deficient in swine. Royal et al. (2013) reported serum concentrations of fentanyl after application of 50 µg $hour^{-1}$ patches in 19 pigs weighing an average of 38.3 kg. The average serum concentrations measured at 24, 48 and 72 hours after application were 0.31 ± 0.07 ng mL^{-1}, 0.21 ± 0.05 ng mL^{-1} and 0.12 ± 0.02 ng mL^{-1}, respectively, thus the authors suggested that therapeutic concentrations were not reached. These concentrations were lower than the Cmax of 0.43 ng mL^{-1} obtained in the present study.

No publication of use of 75 µg $hour^{-1}$ fentanyl TP in swine was found in the literature. In our study, the Cmax of 1.28 ± 0.84 ng mL^{-1} obtained 6 hours after the application of the first patch, was above the reported efficacy reference ranges, suggesting that 75 µg $hour^{-1}$ TP could provide sufficient analgesic relief in swine in the weight range of 25-30 kg.

Wilkinson et al. (2001) applied 100 µg $hour^{-1}$ fentanyl TP in six Yucatán mini pigs weighing 17-

22 kg. The authors proposed a therapeutic range extrapolated from various species of 0.2-3.0 ng mL^{-1}, and reported peak serum concentrations ranging from 0.38 to 0.99 ng mL^{-1}, between 42 hours (n = 5) and 48 hours (n = 1), suggesting that the absorption of transdermal fentanyl at this dose could be effective. Although the animals in the present study had higher body weights, results showed much higher fentanyl concentrations at 6 hours after application of 100 µg $hour^{-1}$ TP (2.16 ng mL^{-1}; n = 3), and the mean plasma concentrations were above the reference value over the following 48 hours. While these observations are limited to a small number of animals, the lack of potential secondary effects related to higher opioid doses suggests that further studies should focus on the 75 µg $hour^{-1}$ and 100 µg $hour^{-1}$ fentanyl TP.

The application of a second 50 µg $hour^{-1}$ or 75 µg $hour^{-1}$ TP provided the unexpected results of serum fentanyl concentrations lower than those obtained with the first patch. A study of the sequential application of opioid TPs in swine has not been reported and further investigations should address this practice in clinical and experimental settings.

In the present study, no serum buprenorphine concentrations were detected in a 28 kg pig after application of a buprenorphine TP (35 µg $hour^{-1}$), neither with the first nor the second application. These results differ from those described by Thiede et al. (2014), who reported maximum concentrations of 0.6 ± 0.1 ng mL^{-1} in five Göttingen mini pigs (weight range 12.6-27 kg), following the application of 30 µg $hour^{-1}$ buprenorphine TP.

The first application of 70 µg $hour^{-1}$ buprenorphine TP to the pigs in this study resulted in detectable serum concentrations of buprenorphine, mean concentration 0.3 ± 0.2 ng mL^{-1} at 33 hours. After application of the second TP, the Cmax was attained much faster, at 12 hours. This possibly represents a more rapid release of buprenorphine, perhaps potentiated by drug accumulation in the subcutaneous fat. However, the results are from only two pigs and the range of concentrations was wide.

The differences between the results reported here and those previously published (Wilkinson et

al. 2001; Malavasi et al. 2005, 2006; Thiede et al. 2014) emphasize the importance of potential confounders. Absorption from a TP can be influenced by poor adherence to the skin, the composition of the skin that will be influenced by the breed and anatomical location and the inter-individual diversity in the kinetics of the molecule. The interscapular area was chosen for TP application in this study because it is a site with limited access by the pig thus reducing the possibility of accidental removal, yet the area provides an adequate space to accommodate the bigger patches. Absorption was not notably affected when compared with other studies (Malavasi et al. 2005, 2006).

A local increase in blood flow will increase fentanyl absorption from a TP, such as may occur when a pig is lying on a patch on a heating pad. This was not an issue with the present study because the TP was applied after surgery.

Excitement should be avoided during handling of the pigs during collection of blood samples. While 3 days for acclimatization may seem short (Swindle & Smith 2013), the pigs in this study quickly adjusted to the investigators and the repetition of handling gestures with apparent minimum of stress and no restraint was needed. Both indwelling catheters (Zanella & Mendl 1992; Lombardo et al. 2010) and vascular access ports (Chuang et al. 2005; Swindle et al. 2005; Ege et al. 2006) are used for repeated blood sampling in swine. The indwelling catheters in this study remained patent for on average 11 out of 13 samples in the first series and on average for 25 out of 26 samples in the second series, with no observable indications of infection over 7 days.

Study limitations include the small sample size and different numbers of animals among the groups, which could influence the statistical analysis and the validation of the observations. Further, the investigators were not blinded to the type of opioid administered during observations of the pigs and that could have influenced the pain scores. No significant differences in the behaviour of the animals before and after ovariectomy were recorded, however the observations

were made every 6 hours and a shorter interval between assessments may have identified some differences. Alternatively, the pain scoring system may not be a sufficiently sensitive indicator of pain in pigs, particularly considering the lack of serum buprenorphine concentrations in the six ovariectomized pigs administered 35 μg $hour^{-1}$ TPs. Further studies using more detailed pain assessment in pigs with TPs are necessary.

Conclusion

Serum concentrations of fentanyl and buprenorphine were measured after transdermal patch application; fentanyl at 50, 75 and 100 μg $hour^{-1}$, and buprenorphine at 35 and 70 μg $hour^{-1}$. The serum concentrations of fentanyl after doses of 75 or 100 μg $hour^{-1}$ to pigs weighing 25-30 kg were within the target range suggested to provide analgesia for up to 48 hours. No vomiting or diarrhoea was observed and the pigs continued to gain weight.

The absence of serum concentrations of buprenorphine following the application of 35 μg $hour^{-1}$ patches prevents us from recommending its use in pigs in this weight range. Owing to the broad variations encountered in serum concentrations following the application of 70 μg $hour^{-1}$ buprenorphine patches, additional studies are needed before recommending its use as sole method of pain control in the laboratory pig.

Sequential applications of fentanyl or buprenorphine TP did not provide reliable serum concentrations; and these results do not support this method of TP use for long-term analgesia in the pig. Further pharmacokinetics studies and analgesiometric tests are required to confirm the clinical adequacy of TP in swine.

Acknowledgements

This study was funded by the Department of Anaesthesiology, Pharmacology and Intensive Care, University of Geneva, Switzerland. The authors thank Mrs Sylvie Roulet, Mr Manuel Costa, Centre Medical Universitaire, University of Geneva, Geneva, Switzerland and Dr Mustafa Cikirikcioglu, Geneva University Hospitals and University of Geneva, Geneva, Switzerland for their invaluable logistical help.

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SOL: project development, experimental procedure, data collection, data analysis and interpretation, preparation and revision of manuscript. WH: project development, preparation and revision of manuscript. YD: laboratory testing and analysis, preparation of manuscript. ZP: statistical design and analysis, preparation of manuscript. PK: project design and development, preparation and revision of manuscript.

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Table 1 Time (T_{max}) to peak serum concentrations (C_{max}) of fentanyl after application of transdermal patches of three dosages to pigs in the first series (1) and the second series (2).

Patch dosage (µg hour^{-1})	$T_{max}1$ (hours)	C_{max} 1 (ng mL^{-1})	$T_{max}2$ (hours)	C_{max} 2 (ng mL^{-1})
50	30	0.43 ± 0.18 (*n* = *6*)	30	0.26 ± 0.02 (*n* = *6*)
75	6	1.28 ± 0.84 (*n* = *3*)	36	0.45 ± 0.08 (*n* = *2*)
100	6	2.16 ± 1.46 (*n* = *3*)	NA	NA

n = number of pigs; NA, no patch applied.

Appendix 1 Pain assessment

Recorded behaviour	Score	Description
General behaviour	+ to +++	Responds to handler, alert, aggressive, depressed, excited
Active	+ to +++	Interaction with handler, with surroundings
Normal gait	Yes/No	Relaxed posture, arched back
Walking	Yes/No	Ambulates normally, lameness
Vocalizations	Yes/No	Abnormal vocalizations, groaning
Vomit	Yes/No	Vomit present in pen
Defecation	Yes/No	Normal/diarrhoea
Local sign of pain	Yes/No	Redness, bleeding, swelling, reaction to palpation

+, low pain; +++, high pain

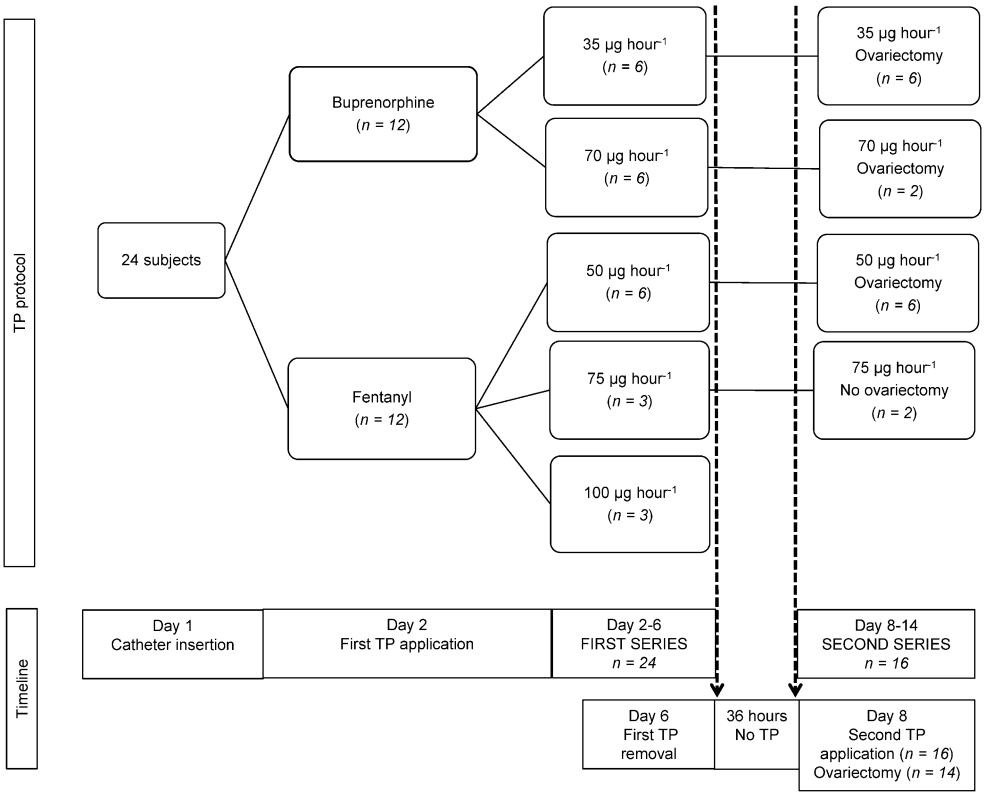
Figure legends

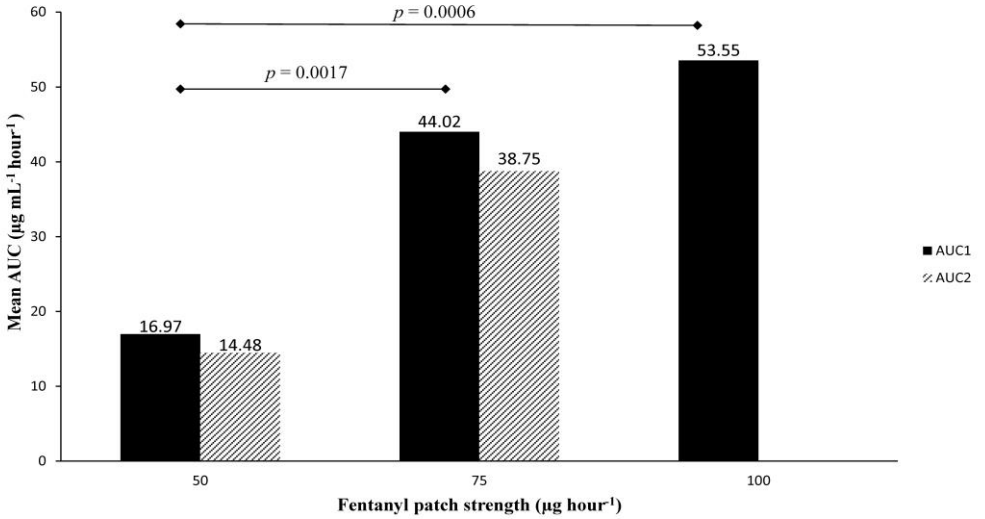
Figure 1 Timeline and subject randomization for application of fentanyl and buprenorphine transdermal patches (TP) in 24 Yorkshire gilts weighing a mean of 27.8 kg. *n* = number of pigs.

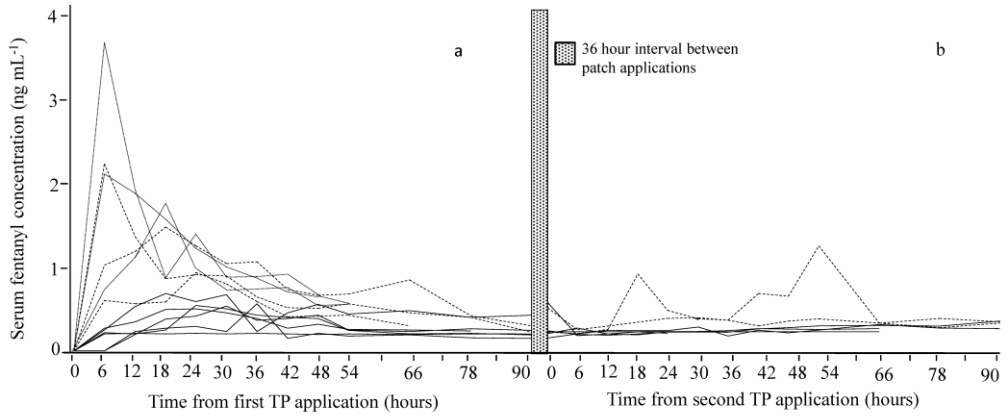
Figure 2 Serum fentanyl concentrations expressed as area under the curve (AUC) of different strengths of fentanyl patches applied to pigs: AUC1 is the mean AUC over 0-54 hours in the first series for patches 50 µg mL^{-1} (*n* = 6), 75 µg mL^{-1} (*n* = 3) or 100 µg mL^{-1} (*n* = 3), and AUC2 is the mean AUC over 0-66 hours in the second series for patches 50 µg mL^{-1} (*n* = 6) and 75 µg mL^{-1} (*n* = 2).

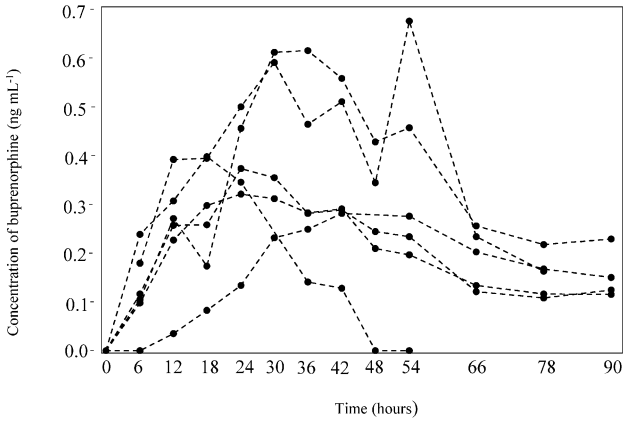
Figure 3 Individual profiles of the serum fentanyl concentrations (ng mL^{-1}) in pigs for the first and second transdermal patch (TP) application. For the first TP: 50 µg $hour^{-1}$ (—) (*n* = 6), 75 µg $hour^{-1}$ (--) (*n* = 3), 100 µg $hour^{-1}$ (···) (*n* = 3). Eight pigs were administered a second TP 36 hours after the first patch was removed: 50 µg $hour^{-1}$ (*n* = 6) and 75 µg $hour^{-1}$ (*n* = 2).

Figure 4 Individual profiles for six pigs of serum buprenorphine concentrations (ng mL^{-1}) before and after application of the first 70 µg $hour^{-1}$ patch. Values below the 0.05 ng mL^{-1} limit of quantification are not indicated.


TP protocol
24 subjects
Buprenorphine
(n = 12)
Fentanyl
(n = 12)
35 µg hour-1
(n = 6)
70 µg hour-1
(n = 6)
50 µg hour-1
(n = 6)
75 µg hour-1
(n = 3)
100 µg hour-1
(n = 3)
35 µg hour-1
Ovariectomy
(n = 6)
70 µg hour-1
Ovariectomy
(n = 2)
50 µg hour-1
Ovariectomy
(n = 6)
75 µg hour-1
No ovariectomy
(n = 2)
Timeline
Day 1
Catheter insertion
Day 2
First TP application
Day 2-6
FIRST SERIES
n = 24
Day 8-14
SECOND SERIES
n = 16
Day 6
First TP
removal
36 hours
No TP
Day 8
Second TP
application (n = 16)
Ovariectomy (n = 14)


p = 0.0006
p = 0.0017
53.55
44.02
38.75
16.97
14.48
60
50
40
30
20
10
0
Mean AUC (µg mL-1 hour-1)
50
75
100
Fentanyl patch strength (µg hour-1)
AUC1
AUC2


Serum fentanyl concentration (ng mL-1)
4
3
2
1
0
a
36 hour interval between patch applications
b
0 6 12 18 24 30 36 42 48 54 66 78 90
Time from first TP application (hours)
0 6 12 18 24 30 36 42 48 54 66 78 90
Time from second TP application (hours)


Concentration of buprenorphine (ng mL-1)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
6
12
18
24
30
36
42
48
54
66
78
90
Time (hours)

Acknowledgements

My heartfelt thanks to Dr. med. vet. Peter Kronen and Prof. Dr. Walid Habre for their mentoring and support, this project would have not been possible without your help.

My most sincere thanks to Prof Dr. med. vet. Brigitte von Rechenberg for her invaluable and generous guidance.

My deep gratitute to Dr. Mustafa Cikirikcioglou, Mme Sylvie Roulet and Mr Manuel Costa for their friendship and invaluable logistical help.

My sincere thanks to Ms Silvia Kaufmann, for her patience and administrative guidance through this study.

Last but not least, my immense gratitude to my husband, Ed, for being there at each step of the way, with love, good advice and endless support.

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